# SPECIES IDENTIFICATION AND COMPARISON OF 5% NATAMYCIN AND 0.15% AMPHOTERICIN WITH 5% NATAMYCIN AND 0.03% CHLORHEXIDINE IN FUNGAL CORNEAL ULCERS

REGIONAL INSTITUTE OF OPHTHALMOLOGY AND GOVERNMENT OPHTHALMIC HOSPITAL

MADRAS MEDICAL COLLEGE,
CHENNAI.

DISSERTATION FOR

M.S. BRANCH III OPHTHALMOLOGY

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY,
CHENNAI, INDIA.
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# CERTIFICATE

This is to certify that **Dr.PRIYA.S,** Post Graduate student in M.S Ophthalmology, at Regional Institute of Ophthalmology and Government Ophthalmic hospital attached to Madras Medical College, Chennai, carried out this dissertation on **"SPECIES IDENTIFICATION AND COMPARISON OF 5%NATAMYCIN AND 0.15% AMPHOTERICIN WITH 5% NATAMYCIN AND 0.03% CHLORHEXIDINE IN FUNGAL CORNEAL ULCERS"** under my direct guidance and supervision during the period from May 2006 to March 2009.

This dissertation is submitted to the TamilNadu Dr.MGR Medical University, Chennai in partial fulfillment of award of M.S. Degree in Ophthalmology.

**Prof. Dr. K.VASANTHA,** M.S,FRCS.
Chief, Cornea Services,
RIO - GOH,
Chennai - 8.

**Prof. Dr. M. RADHAKRISHNAN,**M.S,DO.
Director & Superintendent,
RIO - GOH,
Chennai - 8.

**Prof.Dr. T. P. KALANITI, M.D.,**
DEAN,
Madras Medical College, Chennai.

# DECLARATION

I, **Dr. PRIYA.S ,** solemnly declare that the dissertation titled **"SPECIES IDENTIFICATION AND COMPARISON OF 5%NATAMYCIN AND 0.15% AMPHOTERICIN WITH 5% NATAMYCIN AND 0.03% CHLORHEXIDINE IN FUNGAL CORNEAL ULCERS"** has been prepared by me. This is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the requirement for the award of M.S. Ophthalmology, degree Examination to be held in March 2009.

Place: Chennai

Date:

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## INTRODUCTION

Over one hundred fungal species, representing a wide spectrum of filamentous fungi, yeasts and dimorphic organisms have been reported as corneal pathogens.

Due to a large agrarian population and environmental factors, fungi contribute largely to the environmental list of infectious intruders of the cornea[4]. Review of Indian literature reveals that aspergillus and fusarium are the common fungi causing fungal keratitis.

Schonlein, in 1837 had shown that fungi are capable of producing disease.

Remak, in 1945, successfully transferred fungi from apple to his own skin. Gruby had shown in1841 that favus mould was the culprit for alopecia which was confirmed by Sabourad in 1892.

In 1856, Virchow coined the term mycosis.

In 1897, Leber reported Aspergillus as the cause of corneal ulcer with hypopyon.

Several thousands of species of fungi have been identified. About 50 species are capable of producing disease in humans and animals. Over 70 Genera representing a wide spectrum of filamentous fungi and yeasts forms have been identified in fungal keratitis.

Fungal keratitis remains a diagnostic dilemma and therapeutic challenge to the ophthalmologists, because of its tendency to mimic other types of corneal stromal inflammation.

## GENERAL MYCOLOGY

## CLASSIFICATION:

Fungi are a group of organisms that lack chlorophyll and reproduce sexually or asexually by formation of conidia or by budding.

In contrast to bacteria they are Eukaryotic and have a cell membrane rich in sterol (ergosterol).

Fungi are primitive, non-motile, plant like structures that may grow as unicellular organisms called YEASTS and multicellular filamentous structures called MOULDS.

## MORPHOLOGICAL CLASSIFICATION:

### 1) Moulds/Filamentous Fungi:

These are multicellular organisms which produce long branching hyphae. The hyphae are septate or non septate. Most cases of fungal keratitis are caused by fungi with septate hyphae. Reproduce by means of spores produced by asexual cell division or as a result of sexual reproduction.

**2) Yeasts:**

These are unicellular organisms and round in shape. They reproduce by budding, an asexual process and form pseudohyphae under reduced oxygen tension in the tissue. The pseudohyphal phase is the most invasive and virulent phase.

**3) Yeasts Like Fungi:**

They grow partly as yeast and partly as elongated cells resembling hyphae. The latter form a pseudomycelium.

**4) Dimorphic Fungi:**

These can occur as filaments or as yeast depending on the condition of growth. In host tissues or cultures at $37^0$C, they occur as yeasts while in the soil and in cultures at $22^0$C, they appear as moulds.

**CLASSIFICATION ACCORDING TO SEXUAL SPORES:**

**1) Zygomycetes:**

They are septate filamentous fungi and are rare human corneal pathogens. Genera of this class are mostly associated with orbital phycomycosis.

**2) Ascomycetes:**

They are septate fungi and contain spores in the sacs or asci. Corneal pathogens representing this class include the Genera Aspergillus and Penicillium.

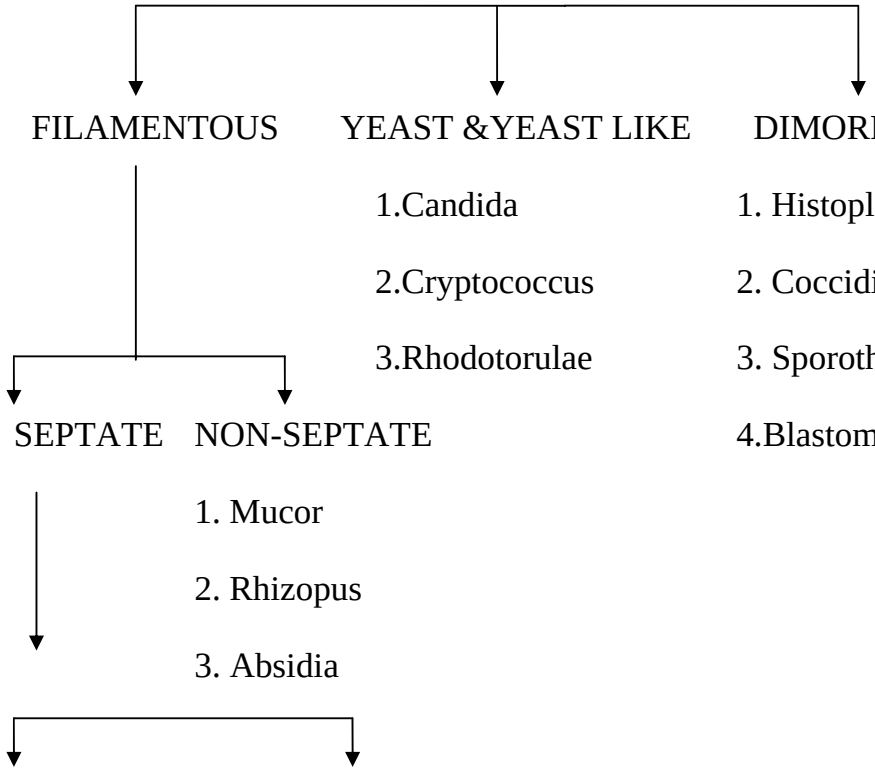
**3) Basidiomycetes:**

These are septate fungi and have sexual spores in club shaped structures called BASIDIA. Mushrooms and plant rusts belong to this class.

**4) Deutromycetes or Fungi imperfecti:**

Most of human corneal pathogens belong to this class. The term imperfecti means lack of sexual spores.

# FUNGI


FILAMENTOUS
YEAST &YEAST LIKE
DIMORPHIC
1.Candida
2.Cryptococcus
3.Rhodotorulae
1. Histoplasma
2. Coccidiodes
3. Sporothrix
4.Blastomycetes
SEPTATE
NON-SEPTATE
1. Mucor
2. Rhizopus
3. Absidia
Non-Pigmented
(Moniliacea)
1. Fusarium
2. Aspergillus
3. Acremonium
4. Penicillium
5. Petricllidium boydii
6. Geogrichum
7. Myrathecum
8. Volutella
9.Cylindro carpon
Pigmented
1. Curvularia
2. Melanconliaces
3. Alternaria
4. Drechslera
5. Cladosporum
6. Phialophora

**MORPHOLOGY:**

Fungi are plant like organisms but lack chlorophyll. So they are saprophytic (living on dead or dying organic matter).

Cell wall is an important structure of fungus which defines it. It is composed of ,

- Polysaccharide
- Lipids

**Polysaccharide(80-90%):**

It is usually cellulose or chitin.

**Lipids:**

Ergosterol is the sterol of fungal cell wall instead of cholesterol in animal cell wall. This difference is taken advantage by currently used antifungal agents.

The fungal cell wall controls the influx and outflux of ions into the fungal protoplasm. The antifungal agents act by altering this mechanism.

## EPIDEMIOLOGY:

### Habitat:

1. Candida is very rare in India as causative organism except in eyes predisposed to it.

2. In Northertn part of India, Nepal, Coastal Karnataka, Aspergillus species are found to be frequently involved whereas in Southern part of India, Fusarium species are reported as the leading cause of keratitis (Dos Times September 2003,Vol.9/No.3 M.Srinivasan, Aravind Eye Hospital, Madurai).

In general Aspergillus species are common in India as shown by many studies.

Fungi common in cooler parts of world is candida whereas Aspergillus and Fusarium are common warmer climates[2].

Fungi rarely infect intact cornea. They are opportunistic pathogens. They infect when host immunity is deranged either locally or when systemic immune deficiency exists.

Fungi are ubiquitous, their light spores produced in huge numbers are widely disseminated and have remarkable ability to germinate and grow on almost any organic substance.

Fusarium species are primarily plant pathogens and keratomycosis caused by them are common in agricultural workers.

Candida species are endogenous opportunistic pathogens that normally reside in or on mammalian host.

Farmers and others involved in agricultural works are more commonly affected[4]. Others include manual labourers and quarry workers. Most often history of injury with organic substance can be obtained[4].

**Microbiology of corneal fungal pathogens:**

**1. Aspergillus:**

They are the easiest to identify if the spores are present. The conidiospores with its swollen terminal end (vesicle) surrounded by flask shaped sterigmata each of which produce long chains of coccoid conidia that radiate out from the vesicle.

The dichotomous branching nature of the hyphae is also diagnostic.

**Cultural Characteristics:**

In Aspergillus, two species are important.

Asp.fumigatus colonies are white at first but as spores are produced, the colonies become velvety green owing to the pigmentation of the conidia. It can tolerate high temperatures and will grow at $50^0$C invitro.

Asp.niger colonies are also white during the initial growth phase but turn completely black as they sporulate.

**2) Fusarium:**

These are characterized by the distinct spores referred to as macroconidia and in some by small coccoid spores known as microconidia.

The main identifying feature of Fusarium is the large banana shaped conidia which are produced on short lateral hyphae – conidiospores. A small cluster of conidia is produced at the tip of each conidiospore.

The aggregates of microconidia bear resemblance to a bunch of bananas. The conidia often have transverse separation.

**Cultural Characteristics:**

The colonies are usually white in the early stages but often acquire buff coloration. As the colonies mature, a variety of coloured pigments ranging from yellow to red purple are produced.

The pigments are seen on the undersurface of the colony. This is known as reverse pigmentation.

**1. Acremonium:**

This species is devoid of macroconidia, produce only microconidia. The spores are produced at the end of lateral hyphae that develops into conidiospores with one or more oval conidia, each of which has a single septum.

The conidiospores produce a sticky mucilaginous substance that holds the conidia together.

**Cultural Characteristics:**

Young colonies of Acremonium are compact and moist but rapidly develop the typical wooly mould like appearance with an overgrowth of white aerial mycelia. Pigments vary from grey to white and best appreciated by looking at the undersurface.

**3) Yeast Forms:**

The yeast form is represented by Candida species. The presence of budding yeast in a corneal scraping is almost diagnostic of Candida.

It produces pseudohyphae and true hyphae and both the yeast forms and hyphal forms are seen in corneal scrapings.

The hyphal form is considered invasive which is the most virulent stage.

**Cultural Characteristics:**

They are distinct from the colonies of filamentous fungi and may resemble bacterial colonies when seen on blood agar. The typical Candida colonies are grown on Sabourad agar which are white and opaque with smooth, flat, round contour and a pasty soft consistency. The colonies are few mm in diameter after 48 hrs incubation at $30^0$C which will continue to grow upto 0.5cm.

When grown on blood sugar at $35^0$C, Candida colonies are minute and easily mistaken for micrococcus colonies.

The colonies have typical yeasty odour which is a useful identifying feature.

**PATHOGENICITY:**

The pathogenic mechanisms of fungi include,

1. Direct physical damage caused by invasion and growth of fungal elements.

2. Damage resulting from infiltrating leucocytes.

3. Damage produced by fungal toxins and enzymes.

In corneal fungal infections, clinical manifestations may occur as quickly as 24-48 hrs or may be delayed for 10-20 days, potentially allowing the extensive fungal replication before deduction by the host.

Infiltration of the host leucocytes is an important component of the corneal damage produced in keratomycosis. Ring abscesses composed

Of PMN leucocytes, plasma cells and rare eosinophils around the fungi are characteristics. (Naumann,G,Green and Zimmermann).

Leucocytes are also seen in areas of corneal damage without fungi.(Fonus A et al.,)

Fungal hyphae are large enough to preclude ingestion by neutrophils, however neutrophils are known to destroy fungal hyphae and probably also damage surrounding tissue via frustrated phagocytosis with consequent extracellular release of lysosomal enzymes and oxygen metabolites.(Diamond et al.,)

**Immunological Aspects:**

Cell mediated immunity plays an important role in protecting the host from fungi. Diamond et al., have shown that T-cell mediated immunity is responsible for this protection. They also showed that neutrophils cause fungal hyphal death by attaching throughout the surface of hyphae even in the absence of complement or antibody. This fungicidal effect of the neutrophils is attributed to the myeloperoxidases and hydrogen peroxide they produce.

## CLINICAL FEATURES:

### Keratitis caused by filamentous fungi:

1. More common in young individuals involved in outdoor activities.
2. There is always a history of trauma with vegetable matter.
3. Incubation period is 24-48 hrs. It may involve any area of the cornea.
4. No evidence of prior ocular surface disorder like dry eyes, herpes or neuroparalytic keratitis is present.

## CHARACTERISTIC FEATURES:

1. Greyish white infiltrate with hyphate margins elevated above the surface of the cornea, surrounded by satellite lesions.
2. Ulcer base may have a dry texture.
3. Ulcer margins are irregular and elevated; may demonstrate irregular fine, linear infiltration branching into the surrounding cornea.
4. Satellite lesions are discrete, stromal abscesses that surround the ulcer and are separated by clear cornea.

5. Clear endothelial plaques are seen composed of inflammatory cells.

6. Immune ring composed of antigen-antibody complex.

7. Hypopyon is a commonly associated feature even with a small ulcer but is not specific.

**Keratitis caused by non-filamentous fungi:**

This occurs commonly and exclusively in eyes with pre-existing corneal surface abnormalities like keratitis sicca, neuroparalytic keratitis, herpes affected eyes.

**CHARACTERISTIC FEATURES:**

1. Ulcer usually occurs at the area of exposure; at the junction of superior 2/3 and inferior 1/3.

2. Keartitis is more localized, may have an expanding, small discrete infiltrate.

3. No hyphate margins and edges not feathery.

4. Satellite lesions are not usually seen.

## LABORATORY INVESTIGATIONS:

### Corneal Smear:

It is done with platinum loop or Bard parker 15 blade.

Smears are taken using topical anaesthetic from,

a. Lid margins

b. Conjunctival sac

c. Ulcer margins and bed

Smears are made on precleaned glass slides and fixed immediately with 5% methanol.

One slide is held for reserve and others are processed for

a. Grams stain

b. Giemsa stain

c. PAS stain

d. Grocott Gomori Methenamine silver stain

1) **Grams and Giemsa Stain:**

It selectively stains the fungal protoplasm. Cell walls are not stained. Most fungi appears gram positive.

Giemsa stain impart a purplish blue colour to the corneal fungal pathogen.

2) **Grocott Gomori Methenamine Silver Stain:**

Most selective method for identifying fungal elements in tissue. But it is costlier and technique is cumbersome.

It depends on reduction of silver by the oxidized carbohydrate components of the fungal cell wall which stains it black.

A light green counter stain renders a pale transparent background against which the hyphae may be more easily recognized.

3) **PAS reagent:**

In tissue and cytological preparations, the fungi can be demonstrated by PAS reagent.

Hydrolysis and oxidation of cell wall polysaccharides occurs with PAS. The hyphae stain bright red.

4) **Calcoflour Stain:**

It is more sensitive than KOH but it requires special fluorescent microscope.

5) **KOH Mount:**

Fungi can be detected by wet mount with 10-20% KOH. It is less reliable when only few hyphae are present. Its sensitivity is low that only 1/3 of the fungal keratitis become positive by this method.

KOH dissolves cells and debris and retains only fungal elements.

Disadvantages of KOH mount:

- No direct staining
- False positive artefacts are common

**6) Acridine orange fluorescein technique:**

With fluorescein microscope, a brilliant yellow orange hue is seen against a dark background.

**CULTURE:**

Usual ‘C’ streak method is followed on agar plates taking multiple scraping using kimuras spatula or platinum loop.

**SELECTION OF MEDIA:**

The media should enable the recovery of fungi as well as aerobic and anaerobic bacteria. Standard media used are,

a. Blood agar

b. Chocolate agar

c. Sabouraud’s medium

d. Thioglycolate broth

**Blood Agar:**

This will support the growth of most fungi as well as bacteria. Two plates are inoculated , one kept at room temperature for fungal growth and the other at body temperature.

**Sabouraud's Medium:**

It consists of dextrose and pentone with 50 mg of gentamycin to inhibit bacterial growth but should not contain cycloheximide which might inhibit some saprophytic fungi.

**Procedures For Inspecting Culture Media:**

They are inspected daily, microscopic evidence of growth usually occur in 2-3 days, may occur within 24 hours.

For cultures to be said negative they are inspected for atleast 2 weeks.

**Liquid Brain-Heart Infusion Broth:**

This is a liquid media. It is sometimes used as an adjunct to the solid media.

BHI offers excellent recovery of fungi but recovery declines at 37˚C.

**Special Media:**

a. Potato Dextrose Agar

b. Cornmeal Agar

c. Czapek-Dox Agar.

**Confirmation Of Microbiological Evidence:**

a. Presence of fungal elements in corneal smear

b. Growth in ‘C’ streaks on solid media

**Additional Fungal Identification Tests:**

a. Biochemical test

b. Germ tube production

c. Immunodiffusion

d. Counter immunelectrophoresis

e. Latex agglutination

f. Crossed electrophoresis

g. Elisa

**Differential Diagnosis:**

Fungal ulcers should be differentiated from the bacterial ulcers. Bacterial ulcers, especially the pseudomonas ulcers, are produced early within a short incubation period. Other clinical features and microbiological evidence will enable one to differentiate fungal ulcers from the bacterial ulcers easily.

Acanthamoeba keratitis is worldwide in distribution. It causes an indolent keratitis with exacerbation often following trauma or associated with contact lens wear, or presumed herpes simplex keratitis.

Theodre and co-workers have recently shown the diagnostic value of ring stromal infiltrate which has been present in over 2/3 of the reported cases. On occasion an accompanying iridocyclitis and hypopyon have been observed. Pain is the most prominent symptom.

**PHARMOCOLOGICAL TREATMENT:**

Biology of the fungal tissue has an important bearing on the pharmacological management. Both the yeast and filamentous fungi are in the filamentous form only in the corneal tissue. The hyphal membrane regulates the inflow and outflow of the electrolytes from the fungal cell.

The fungal cell wall sterol is ergosterol while that of mammalian cell wall is cholesterol. Most antifungal agents capitalise on this key difference in the plasma membrane constituents in order to damage fungal cells while minimizing damage to the host cells.

However eradication of fungi is frequently difficult due to,

a. Deeply invasive nature of the infectious process

b. Penetration of the fungus through the cornea into anterior chamber.

Therefore, an effective agent for treating fungal keratitis should exhibit pharmacological properties that include excellent corneal and ocular penetration.

## PHARMACOLOGY OF ANTIFUNGAL AGENTS:

### Amphotericin B:

It is a polyene antibiotic derived from strains Streptomyces nodosus.

### Mechanism of action:

The antibiotic binds to ergosterol present in the cell membranes, increase the permeability of cytoplasmic membranes, thereby permitting leakage of essential intracellular constituents. It is fungicidal at high concentration and fungistatic at low concentration.

### Spectrum of activity:

1. Aspergillus
2. Candida
3. Cryptococcus
4. Fusarium and filamentous fungi

**Routes of administration:**

It can be applied as topical, subconjunctival, intracameral, intravitreal injections.

Topical administration of 0.15% Amphotericin B every 5 min for one hour as loading dose followed by one drop every hour throughout day and every 2-4 hours at night. Since corneal epithelium is an effective barrier to the penetration of Amphotericin B, drug efficacy is reduced if the epithelium is intact.

Ointment forms and collagen shields soaked in Amphotericin B have been shown to be effective in the treatment of fungal keratitis.

**Side Effects:**

On local administration, the following side effects can occur

1. Burning sensation
2. Punctate keratitis
3. Eyelids/conjunctiva:

    - allergic reactions

    - ulceration

- follicular conjunctivitis

- necrosis, nodules and yellow coloration on sc injection

4. Overgrowth of non susceptible organisms

5. Uveitis

6. Delayed corneal wound healing

**Natamycin: (Pimaricin)**

This is a small polyene antibiotic first isolated in 1958.

**Mechanism of action:**

It binds to ergosterol present in the cell membranes, increase the permeability of cytoplasmic membranes, thereby permitting leakage of essential intracellular constituents.

**Spectrum of activity:**

1. Fusarium
2. Aspergillus
3. Acremonium
4. Curvularia

**Regimen:**

One drop of 5% suspension every 5 min for one hour has to be instilled in the conjunctival sac as a loading dose followed by one drop every 1-2 hours. The frequency of application can be reduced to one drop 6-8 times daily after the first 3-4 days. Therapy should be generally continued for 14-21 days until the active fungal infection resolves.

**Side Effects:**

1. Moderately severe conjunctival hyperemia
2. Follicle formation
3. Persistent epithelial ulceration

**CHLORHEXIDINE:**

Chlorhexidine is basically used as a topical antiseptic and surfactant in hydrogel lens solutions. It is also useful as an antimicrobial agent. It is a diguanide. It does not alter the corneal permeability due to two reasons:

1. Its structure is such that it has two positive charges that are separated by a long carbon backbone and it cannot intercalate into a lipid layer.

2. Proteins neutralise the toxicity of chlorhexidine in tear film.

Recently chlorhexidine has been used as a first line of drug in the treatment of fungal corneal ulcers when other antifungal agents are not available[3,5].

**Mechanism of action:**

It acts by attacking and rupturing the cell membranes. It inhibits both cation transport and membrane bound ATP in the cell membrane.

**Side Effects:**

**Conjunctiva:**

1. Punctate keratitis
2. Edema
3. Opacification
4. Vascularisation
5. Decreased endothelial count

**Cornea:**

1. Hyperemia
2. Lacrimation
3. Photophobia
4. Ocular pain
5. Burning sensation

## AIM OF THE STUDY:

1. To identify the species responsible for fungal corneal ulcer.

2. To study the response of fungal corneal ulcers for antifungal agents.

3. To compare the efficacy of 0.15% Amphotericin and 5% Natamycin combination with 5% Natamycin and 0.03% Chlorhexidine in case culture positive fungal corneal ulcers caused by filamentous fungi.

## MATERIALS AND METHODS OF STUDY:

The study was conducted in the cornea clinic of Regional Institute Of Ophthalmology. Patients with corneal ulcer were followed from March 2007 to February 2008. During this period 996 cases of corneal ulcers were treated in the cornea clinic. Out of these 264 cases turned out to be fungal corneal ulcers which was confirmed by both clinical and culture methods.

Out of these 264 culture positive cases, 60 cases were selected for the study. Among the 60 culture positives, 30 cases were treated with 0.15% Amphotericin and 5% Natamycin combination and the remaining 30 cases were treated with 5% Natamycin and 0.03% Chlorhexidine combination.

**COURSE OF THE STUDY:**

When a patient with corneal ulcer was noticed, ophthalmological examination like visual acuity, slit lamp biomicroscopy, staining procedures with fluorescein, syringing of the nasolacrimal duct were done and routine smear studies using the No.15 Bard Parker Blade were done under sterile conditions on precleaned glass slides. In addition to these random blood sugar and urine sugar was done to rule out diabetes mellitus.

- One slide was prepared for KOH mount and the presence of any fungal elements were noted.

- Other smear was stained with Grams stain to identify the associated bacterial pathogens.

- Simultaneously, the corneal scraping was inoculated in Sabouraud's agar and kept at room temperature.

- Those cases whose initial smear studies and clinical features favour fungi as being the cause were randomly treated with 0.15% Amphotericin and 5% Natamycin or 5% Natamycin and 0.03% Chlorhexidine combination and other adjuvant medications such as atropine eye drops.

- The fungal species were later confirmed by growth in culture media.

- Those cases which proved to be negative for fungi but presenting with clinical features of fungal corneal ulcers were again subjected to smear and culture study.

- The cultures were considered negative if no growth was noted after 2 weeks of inoculation.

**Treatment Protocol:**

- Species identification was done with Lactophenol cotton blue for 60 culture positive cases. Out of these 60 cases, 30 cases were treated with 0.15% Amphotericin and 5% Natamycin combination and the remaining 30 cases were treated with 5% Natamycin and 0.03% Chlorhexidine.

- All these cases were followed up for a period of 45 days and the response to medications was assessed.

- Finally cases with gross structural damage and good light perception and projection were taken up for therapeutic keratoplasty.

**ANALYSIS:**

During the period of 1 year, 996 cases of corneal ulcers were treated in the cornea clinic department of RIOGOH, Chennai. Out of these, 264 cases were caused by fungi alone. These cases were identified clinically and by KOH mount and later were confirmed by culture methods.

**CULTURE POSITIVE FUNGAL CORNEAL ULCER:**

| | **Male** | **Female** | **Children** | **Total** |
|---|---|---|---|---|
| **Number** | 153 | 101 | 10 | 264 |
| **Percentage** | 57.95% | 38.25% | 3.78% | |

Male patients accounted for the higher incidence of culture positive fungal corneal ulcer.

**AGE DISTRIBUTION:**

| AGE | MALE | FEMALE | TOTAL | PERCENTAGE |
|---|---|---|---|---|
| 0-15 | 6 | 4 | 10 | 3.78% |
| 15-30 | 31 | 12 | 43 | 16.28% |
| 30-45 | 72 | 22 | 94 | 35.60% |
| 45-60 | 48 | 28 | 76 | 28.78% |
| >60 | 23 | 18 | 41 | 15.53% |

The largest incidence of fungal corneal ulcers were among the age group of 30-45 years and the lowest incidence was among the age group of 0-15 years.

**SEASONAL INCIDENCE:**

| MONTH | FUNGAL CULTURE POSITIVES |
|---|---|
| March | 31 |
| April | 33 |
| May | 28 |
| June | 29 |
| July | 20 |
| August | 19 |
| September | 18 |
| October | 19 |
| November | 16 |
| December | 14 |
| January | 18 |
| February | 19 |
| TOTAL | 264 |

**INJURING AGENT:**

| SI No. | Nature Of Agent | Number | Percentage |
|---|---|---|---|
| 1. | Wooden stick | 10 | 16.66% |
| 2. | Foreign body/sand | 4 | 6.66% |
| 3. | Paddy husk/Leaf | 11 | 18.33% |
| 4. | Thorn | 2 | 3.33% |
| 5. | Cowtail | 2 | 3.33% |
| 6. | Cloth | 2 | 3.33% |
| 7. | Others | 3 | 5.00% |
| 8. | No Agent | 20 | 33.33% |

The common injuring agents were paddy husk/leaf followed by wooden stick injury.

**Nature Of Pretreatment:**

| Nature Of treatment | No. Of Cases | Percentage |
|---|---|---|
| Antibiotics | 10 | 16.66% |
| Native Treatment | 5 | 8.33% |
| No Treatment | 32 | 53.33% |

Antibiotics were the common pretreatment applied by most of our patients.

**Clinical Features:**

| Features | Number | Percentage |
|---|---|---|
| Immune ring | 8 | 13.33% |
| Satellite lesion | 22 | 36.66% |
| Hypopyon | 37 | 61.66% |

**Systemic Diseases:**

1. Diabetics - 4 patients

2. Normal - 56 patients

**Organisms isolated:**

| Organisms | Number | Percentage |
|---|---|---|
| Aspergillus niger | 27 | 45.00% |
| Aspergillus fumigatus | 16 | 26.66% |
| Aspergillus flavus | 5 | 8.33% |
| Fusarium | 10 | 16.66% |

**MORPHOLOGY OF THE ULCER:**

Morphology of the ulcer was assessed using the following criteria.

| S No. | Feature | Non-Severe | Severe |
|---|---|---|---|
| 1. | Rate of progression | Slow,Moderate | Rapid |
| 2. | Size of ulcer | <6mm<br>Superficial 1/3 | >6mm<br>Upto Inner 2/3 |
| 3. | Location | Peripheral | Central |
| 4. | Depth of ulceration | Superficial 1/3 | Upto Inner 2/3 |
| 5. | Perforation | Unlikely | Present/Imminent |
| 6. | Scleral suppuration | Absent | Present |

*Severe if 3 or more are met.

(From IJO, 1994 Agarwal et al)

**Based on above criteria**,

| | **Non-Severe** | **Severe** |
|---|---|---|
| **Number of cases** | 45 | 15 |

**Treatment Protocol:**

| | **0.15%Amphotericin & 5% Natamycin** | **5% Natamycin & 0.03%Chlorhexidine** |
|---|---|---|
| **Number of cases treated** | 30 | 30 |

**RESPONSE TO MEDICATION:**

| | **0.15%Amphotericin & 5%Natamycin** | **5%Natamycin & 0.03%Chlorhexidine** |
|---|---|---|
| **No. Of Cases** | 28 | 28 |

No. of cases taken for TKP inspite of treatment = 4

**DISCUSSION:**

During the period of one year from March 2007 to February 2008, out of 264 fungal culture positives, 57.95% of cases were in male sex. This obvious preponderance of male sex is due to their outdoor activities and are prone for injury[4]. This has been clearly pointed out in an article named Prevention of corneal ulceration by Whitcher .

Most of them belong to low socioeconomic group[4]. 44.31% of the cases were in the age group of above 45 years. This has been proved in a study by Rahman et al[3].

History of injury was present in 66.67% of cases. Corneal injury as a major cause of fungal corneal ulcer has been reported in a study named "Microbial keratitis in South India – Influence of risk factors, climate and geographical variation" conducted by Aravind hospital[2]. In 33.33% of the cases there was no history of trauma.

Out of the 60 cases, 5 patients took native treatment and 10 patients took antibiotics as pretreatment.

Immune ring was present in 13.33% of cases, satellite lesions in 36.66% of cases and hypopyon in 61.66% of cases.

Out of the 60 culture positive cases, 4 were diabetics and the remaining 56 were normal.

Species identification was done for only 60 culture positive cases. Most common organism isolated were Aspergillus niger followed by Aspergillus fumigatus, aspergillus flavus and Fusarium[4].

The size and depth of ulcer plays an important role in deciding the response to treatment. Out of the 60 culture positive cases, 45 cases were presenting as non-severe and 15 cases presented as severe variety.

Out of the 60 culture positive cases, 30 patients were treated with 0.15% Amphotericin and 5% Natamycin combination and the remaining 30 patients were treated with 5% Natamycin and 0.03% Chlorhexidine combination. All these 60 patients were followed for a period of 45 days to assess the response to medication.

28 cases in the first group and 28 cases in the second group responded to the respective combination treatment. The response to treatment was analysed by the symptomatic responses and regression of signs like pain, redness, watering, photophobia, size of the ulcer, depth of ulcer and extension of the infiltration.

SUMMARY:

- In this study conducted from March 2007 to February 2008, 264 culture positive fungal corneal ulcers were identified.
- Males constituted majority of the cases.
- The common age group affected were above 45 years.
- Majority of the ulcers were due to trauma.
- Clinical features like immune ring, satellite lesions and hypopyon were taken into consideration while grading the ulcer staging.
- Species identification was done for 60 culture positive fungal corneal ulcer cases. Most common organism isolated were Aspergillus niger followed by Aspergillus fumigatus, aspergillus flavus and Fusarium.
- These 60 cases were divided into two groups, one group treated with 5% Natamycin and 0.15% Amphotericin and the other group were treated with 5% Natamycin and 0.03% Chlorhexidine.

- The size and depth of ulcer plays an important role in deciding the response to treatment.

- 28 cases in the first group and 28 cases in the second group responded to treatment.

- 4 cases did not respond to treatment and underwent therapeutic keratoplasty.

## CONCLUSION:

In our study, species identification was done for 60 culture positive fungal corneal ulcers. These 60 cases were divided into two groups and one group was treated with 5%Natamycin & 0.15% Amphotericin combination and the other group was treated with 5% Natamycin & 0.03% Chlorhexidine combination. The two groups were followed up for a period of 45 days and the response to combination treatment was assessed. The group treated with 5% Natamycin & 0.03% Chlorhexidine showed equal effectiveness as compared to 5% Natamycin & 0.15% Amphotericin. Hence, we conclude that Chlorhexidine can be used as an antifungal agent when other first line antifungal drugs are not available[3,5].

**TEXTBOOKS:**


1) Grayson's diseases of Cornea, IV Edition, 257-283.

2) Gilbert Smolin – The Cornea,III Edition, 229-253

3) David J Apple, V Edition, 66-67.

4) Kaufman – The Cornea,1988, 217-271.

5) Sir Stewart Duke Elder – Diseases of outer eye, Part I, Vol 8, Page 385-397.

6) Principles and Practice of Ophthalmology – Peyman, Vol I, Page 294-298.

7) Jakobeic – Principles and Practice of Ophthalmology, II Edition, Vol II, Page 906-913.

8) Parson's diseases of the eye , 20th Edition, page 213.


**JOURNALS:**


1) Prevention of corneal ulceration in the developing world: International Ophthalmology Clinics,Vol 42(1) Winter 2002,71-77.

2) Microbial keratitis in South India – Influence of risk factors, climate and geographical variation,Ophthalmic epidemiology, Vol 14, Issue 2, March 2007, 61-69.

3) Rahman MR, Minassian DC, Srinivasan M. Trial of Chlorhexidine gluconate for fungal corneal ulcers. Ophthalmic Epidemiology 1997; 4 : 141-49.

4) Fungal profile and susceptibility pattern in cases of keratomycosis Usha Arora, Aruna Aggarwal, Vijay Joshi Vol. 8 No. 1, January-March 2006.

5) Mycotic keratitis: Susceptibility to antiseptic agents, Michael J. Martin, M. Rezanur Rahman, Gordon J. Johnson, M. Srinivasan and Yvonne M. Clayton, Vol 19, No.9, September1995.

6) M.Srinivasan, Christine A Gonzoles, Celine George et al, Epidemiology and etiology of corneal ulcer in Madurai,South India,BJO, 1997;81,965-971.

7) Jayahar Bharathi et al, Trauma and corneal ulcer, Journal of TNOA; 2002; 43A, Issue 2;77

8) Chander J Sharma A, Prevalence of fungal corneal ulcer in North India; IJO; 1994,22: 207-209.

9) Sharma et al, Eraly diagnosis of mycotic keratitis; Predictive value of KOH, IJO; 1998; 46, 31-35.

**KEYWORDS**

| | |
|---|---|
| LE | LEFT EYE |
| RE | RIGHT EYE |
| TKP | THERAPEUTIC KERATOPLASTY |
| KOH | 10% POATASSIUM HYDROXIDE |
| FB | FOREIGN BODY |
| N | NORMAL |
| SH | SEPTATE HYPHAE |
| CONST.WORK | CONSTRUCTION WORKER |
| ASP. NIG | ASPERGILLUS NIGER |
| ASP.FUM | ASPERGILLUS FUMIGATUS |
| ASP.FLAV | ASPERGILLUS FLAVUS |

**PROFORMA**

Name: Date: Address:

Age: Date of Admission:

Sex: Date of Discharge:

O.P No.: MRD No.:

C.C No.:

Complaints:

Pain/Watering/Redness/Photophobia/Opacity/Defective vision

Duration:

Eye:

Present history:

Past history:

Nutritional deficiency:

Injury:

History of previous surgeries:

TB/Syphilis/Leprosy/Diabetes

Occupational/Present/Family history:

General Examination:

Local Examination:

| | RE | LE |
|---|---|---|
| Visual Axis | | |
| Lids and adnexa | | |
| Conjunctiva | | |
| Cornea | | |
| Depth of involvement | | |
| Anterior chamber: | | |
| Iris | | |
| Pupil | | |
| Lens | | |
| Vision | | |
| Without Glasses: | | |
| With Glasses: | | |

Fundus:

Tension:

Investigations:

Fluorescein:

KOH:

Conjunctival smear:

Gram Stain:

Culture  Bacterial

Fungal

Duct:

Urine  Albumin

Sugar

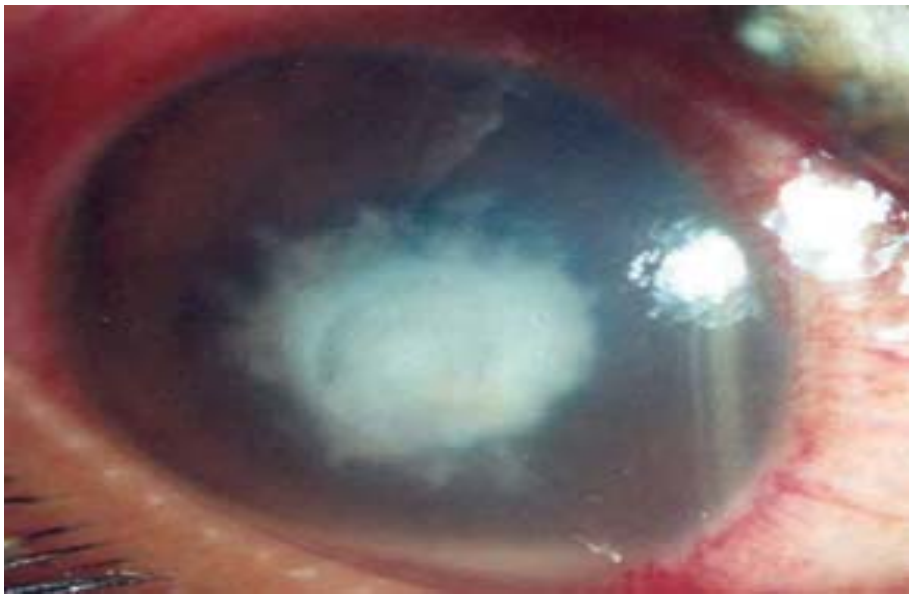
| Symptoms & Signs | D 1 | D 2 | D 3 | D 4 | D 5 | D 6 | D 7 | D 8 | D 9 | D 10 | D 11 | D 12 | D 13 | D 14 | D 15 | D 16 | D 17 | D 18 | D 19 | D 20 | D 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pain** | | | | | | | | | | | | | | | | | | | | | |
| **Redness** | | | | | | | | | | | | | | | | | | | | | |
| **Watering** | | | | | | | | | | | | | | | | | | | | | |
| **Photophobia** | | | | | | | | | | | | | | | | | | | | | |
| **Size of the ulcer** | | | | | | | | | | | | | | | | | | | | | |
| **Depth of ulcer** | | | | | | | | | | | | | | | | | | | | | |
| **Infiltration** | | | | | | | | | | | | | | | | | | | | | |

**LIST OF SURGERIES**

| Sl No. | Name | Age | Sex | IP /OP No. | Diagnosis | Surgery |
|---|---|---|---|---|---|---|
| 1 | Rajammal | 60 | F | 412341 | BE-Immature Cataract | RE-ECCE with PCIOL |
| 2 | Murugan | 65 | M | 423452 | RE-Mature Cataract | RE-ECCE with PCIOL |
| 3 | Muthu | 53 | M | 455678 | LE-Nuclear Cataract | LE-ECCE with PCIOL |
| 4 | Valliammal | 50 | F | 456794 | RE-Chronic Dacryocystitis | RE-DCT |
| 5 | Perumal | 63 | M | 403387 | RE-Immature Cataract | RE-SICS with PCIOL |
| 6 | Premalatha | 20 | F | 465389 | LE-Nasal Pterygium | LE-Pterygium excision with autograft |
| 7 | Devaki | 54 | F | 444560 | RE-Immature Cataract | RE-SICS with PCIOL |
| 8 | Govindaraj | 66 | M | 465612 | RE-Fungal Corneal Ulcer | RE-TKP |
| 9 | Saroja | 55 | F | 485875 | LE-Immature Cataract | LE-SICS with PCIOL |
| 10 | Jyothi | 35 | F | 456784 | RE-Nasal Pterygium | RE-Pterygium Excision with Autograft |

| Sl No. | Name | Age | Sex | IP /OP No. | Diagnosis | Surgery |
|---|---|---|---|---|---|---|
| 11 | Lakshmi | 70 | F | 476605 | LE-Panophthalmitis | LE-Evisceration |
| 12 | Balaraman | 48 | M | 435670 | LE-Fungal Corneal Ulcer | LE-TKP |
| 13 | Alagammal | 50 | F | 467780 | RE-POAG | RE-Trabeculectomy |
| 14 | Ramalingam | 56 | M | 456349 | RE-Full Thickness Corneal Tear | RE-Corneal Tear Suturing |
| 15 | Damodaran | 45 | M | 41506 | RE-Lower Lid Tear | RE-Lid Tear Sutured |
| 16 | Chellammal | 52 | F | 465362 | BE-Immature Cataract | RE-Phaco with PCIOL |
| 17 | Pachiammal | 60 | F | 45723 | RE-Nuclear Cataract | RE-SICS with PCIOL |
| 18 | Ponnusamy | 50 | M | 41276 | LE-Posterior Cortical Cataract | LE-Phaco with PCIOL |
| 19 | Gangammal | 57 | F | 455061 | LE-CACG | LE-Trabeculectomy |
| 20 | Munusamy | 60 | M | 46231 | LE-Immature Cataract | LE-Phaco with PCIOL |

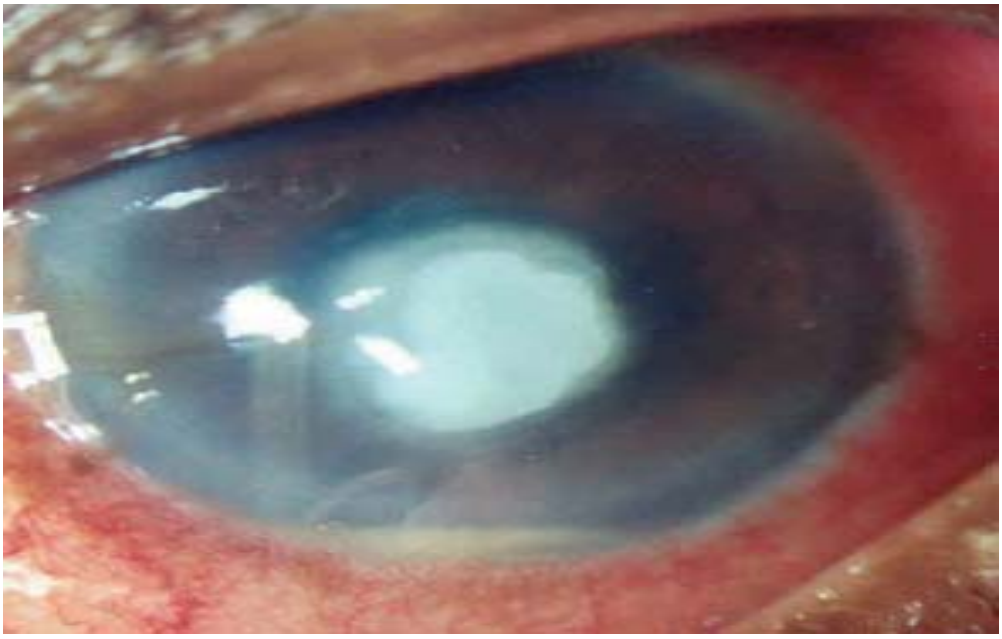
## Fungal Corneal Ulcer With Feathery Margins



## Total Fungal Corneal Ulcer With Stromal Abscess

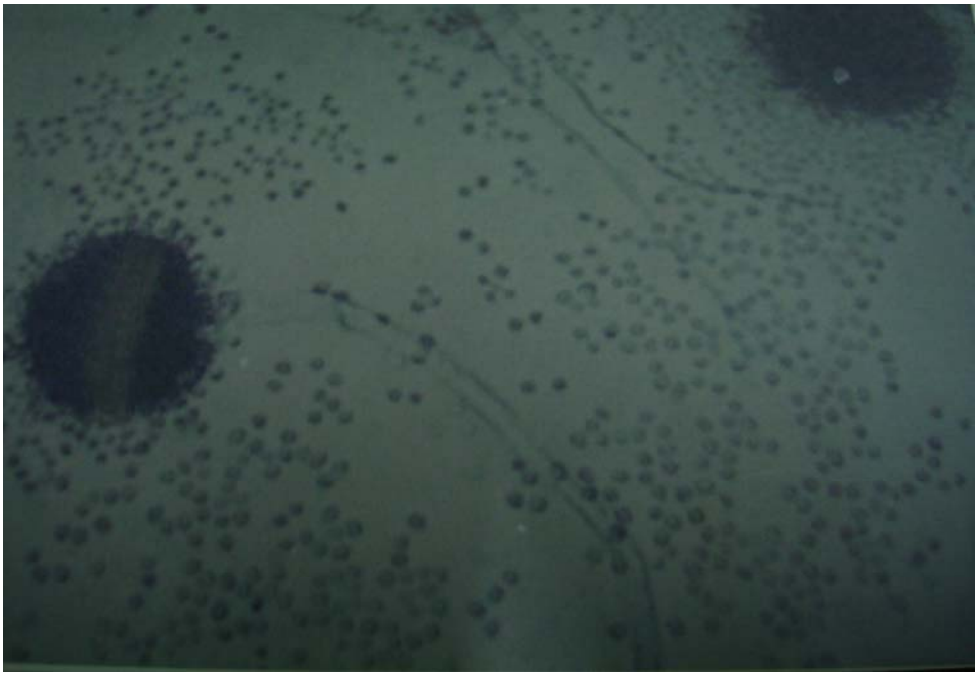
## Boat Shaped Fusarium Spores

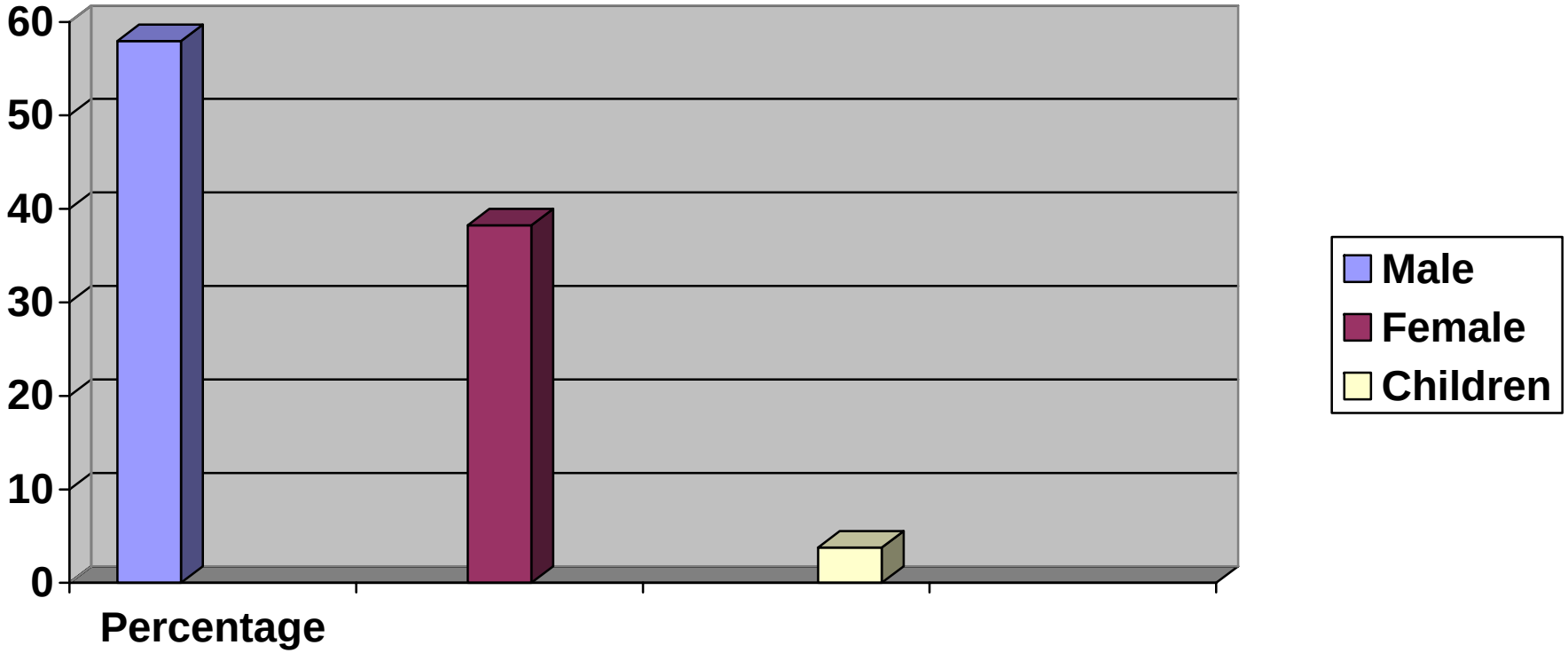
## Fungal Corneal Ulcer With Hypopyon



## Culture Tube Showing Asp niger, Asp fumigatus, Candida albicans

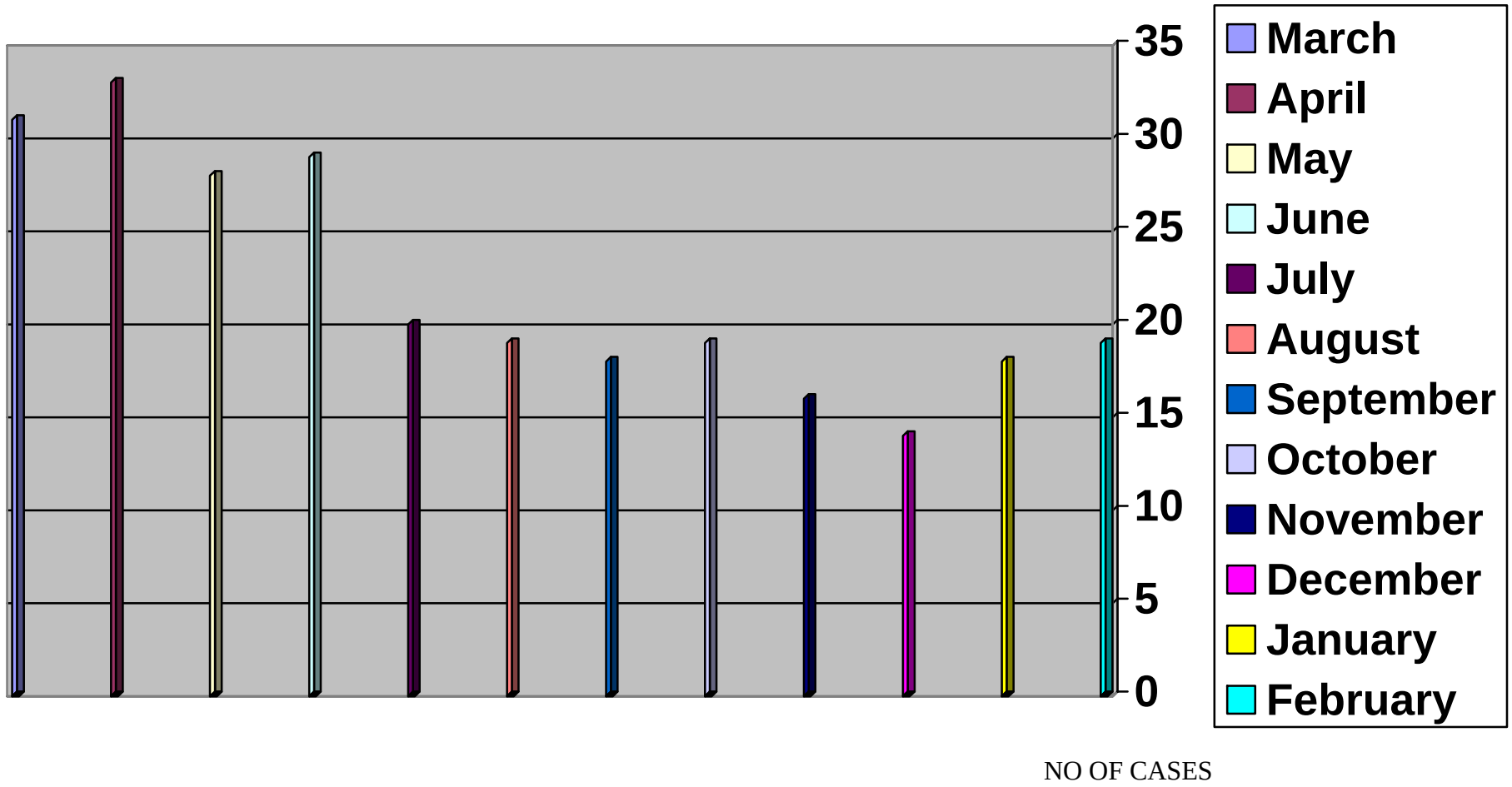
## KOH Mount Showing Septate Hyphae

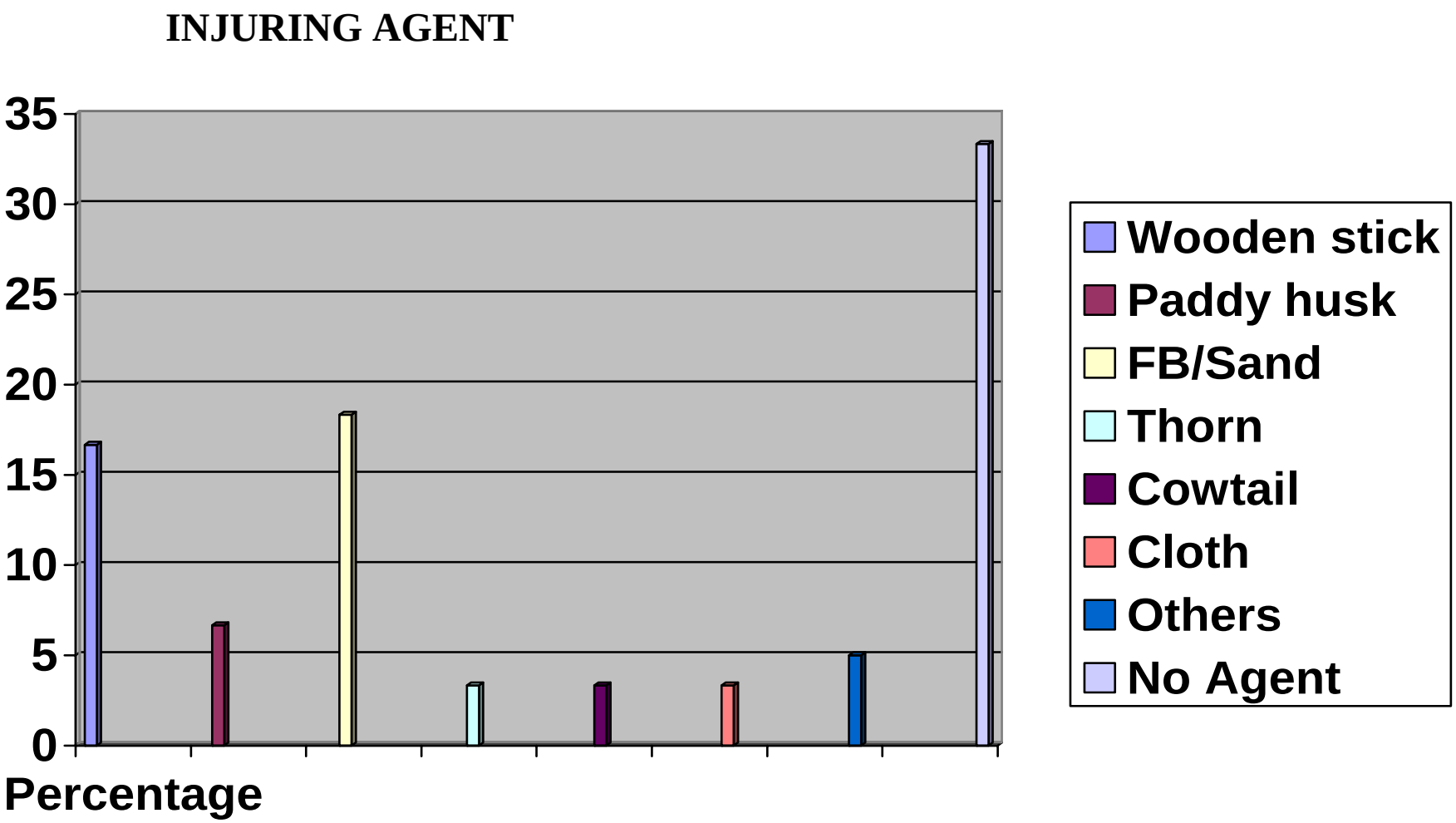




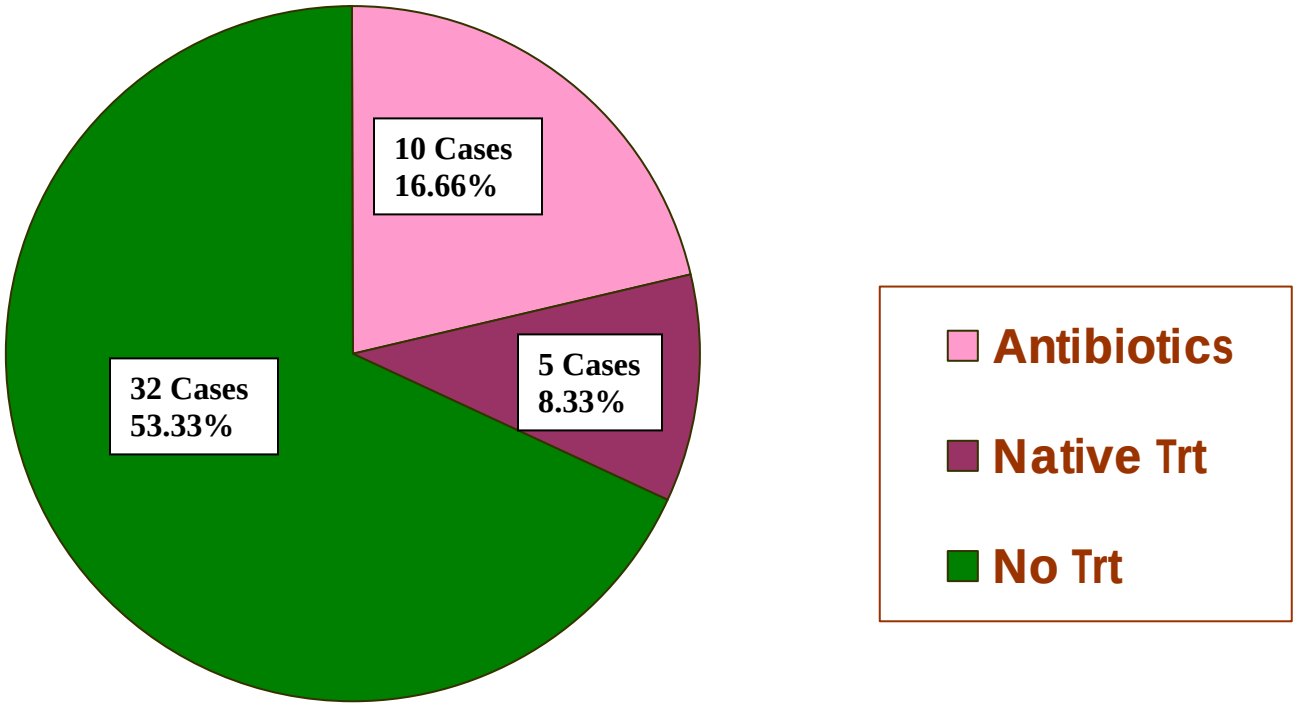

**ANALYSIS DEPENDING ON SEX**

# SEASONAL VARIATION

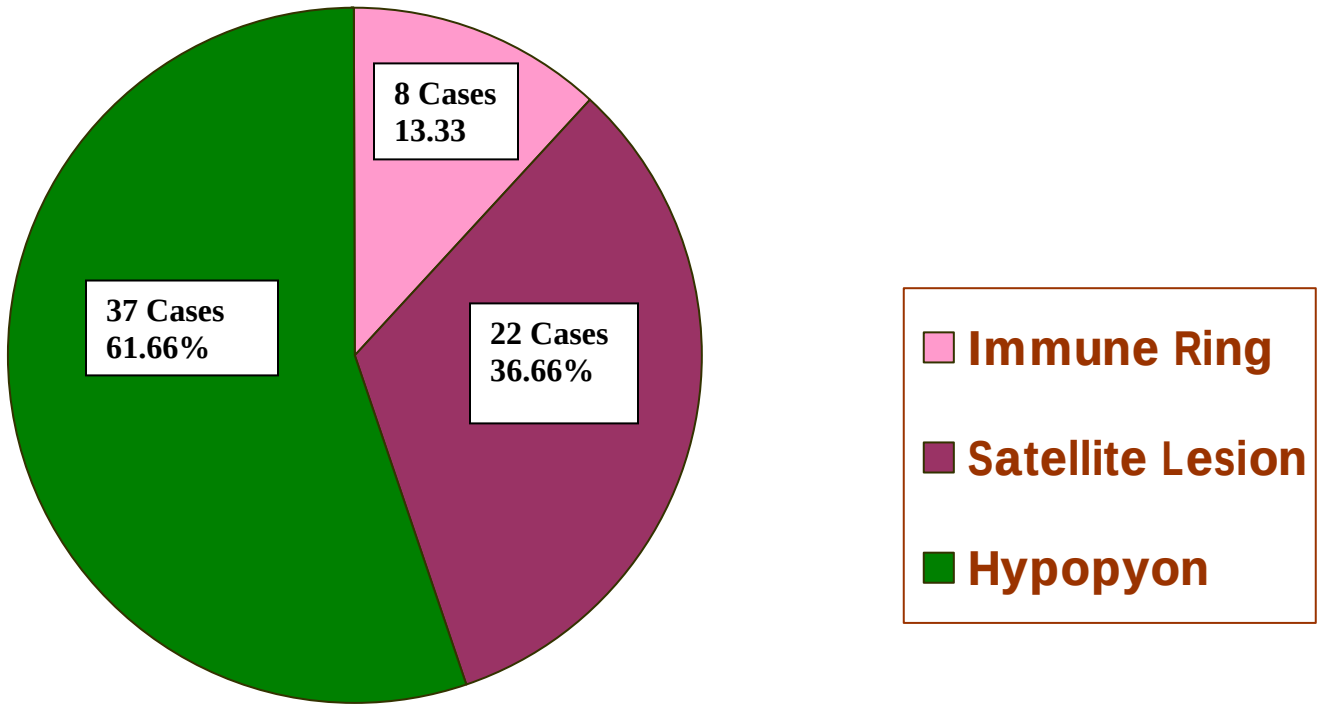



INJURING AGENT
35
30
25
20
15
10
5
0
Percentage
Wooden stick
Paddy husk
FB/Sand
Thorn
Cowtail
Cloth
Others
No Agent

# NATURE OF PRETREATMENT



# CLINICAL FEATURES



## ORGANISMS ISOLATED